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CHROMOSOMAL DATA OF TWO PHOLCIDS (ARANEAE, HAPLOGYNAE): A NEW DIPLOID NUMBER AND THE FIRST CYTOGENETICAL RECORD FOR THE NEW WORLD CLADE

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ABSTRACT. *Mesabolivar luteus* (Keyserling 1891) and *Micropholcus fauroti* (Simon 1887) specimens were collected in Ubatuba and Rio Claro, both in the state of São Paulo, Brazil. *Mesabolivar luteus* showed 2n (♂) = 15 = 14 + X and 2n (♀) = 16 = 14 + XX in mitotic metaphases and 7II + X in diplotenic cells. During late prophase I, all bivalents presented a ring shape, evidencing two chiasmata per bivalent. In this species, some diplotenic cells appear in pairs, maybe due to specific characteristics of the intercellular bridges. The metaphases II showed n = 7 or n = 8 = 7 + X chromosomes. *Micropholcus fauroti* evidenced 2n (♂) = 17 = 16 + X in spermatogonial metaphases and 8II+X in diplotenic cells, with only one chiasma per bivalent, contrasting with *M. luteus*. In both species, all chromosomes were metacentrics. The sexual chromosome X was the largest element and appeared as a univalent during meiosis I. These are the first cytogenetical data for the genera *Mesabolivar* and *Micropholcus*. Additionally, *M. luteus* is the first chromosomally analyzed species of the New World clade and the observed diploid number for *M. fauroti* had not yet been recorded in Pholcidae.

Keywords: Arachnida, Meiosis, chromosomal morphology, spider, diplotene pair

Pholcids are small to medium sized spiders, with total length ranging from 1–15mm, usually with six or eight eyes, and legs several times longer than the body length. Specimens are found in low and high elevations, forests and deserts, leaf litter and tree canopies. There are several synanthropic species with cosmopolitan distribution. These characteristics taken together make the family Pholcidae Koch 1851 the most diverse among the haplogyne group, comprising 75 extant genera and 866 extant species (Huber 2000, 2005).

According to the cladogram proposed as a working hypothesis by Huber (2000) for the New World pholcids, the family is strongly supported as a monophyletic group and is divided into four clades: "ninetines", "pholcines" (*Metagonia* Simon 1893 + *Pholcus* group sensu Huber, 1995), "holocnemines" (*Holocnemus* group sensu Timm, 1976 + *Artema* Walckenaer 1837 + *Physocyclus* Simon 1893 + *Priscula* Simon 1893) and "New World clade". The latter includes most of the genera and is the only one that is exclusive for the New World. However, Huber (2000) himself pointed to "ninetines" and "holocnemines" as questionable monophyletic groups.

Despite the high number of Pholcidae species, only nine species (1%) of five genera have been chromosomally analyzed, i.e., "pholcines": *Pholcus crypticolens* Bösenberg & Strand 1906, 2n (♂) = 24 = 22 + X_1X_2 (Suzuki 1954); *Pholcus manueli* Gertsch 1937 (under *Pholcus affinis* Schenkel 1953), 2n (♂) = 25 = 24 + X (Wang et al. 1997); *Pholcus phalangioides* (Fuesslin 1775), 2n (♂) = 24 = 22 + X_1X_2 (Rodríguez-Gil et al. 2000) and *Spermophora senoculata* (Dugès 1836) (under *Spermophora meridionalis* Hentz 1841, misspelled as *Spermaphora meridionalis*), 2n (♂) =? =? + X_1X_2 (Painter 1914), and "holocnemines": *Artema atlanta* Walckenaer 1837 (misspelled as *Artema atlenta*), 2n (♂) = 32

= 30 + X_1X_2 (Parida & Sharma 1987; Sharma & Parida 1987); *Crossopriza lyoni* (Blackwall 1867), 2n (♂) = 27 = 26 + X (Bole-Gowda 1958), 2n (♂) = 25 = 24 + X (Srivastava & Shukla 1986), 2n (♂) = 24 = 22 + X_1X_2 (Sharma et al. 1959) and 2n (♂) = 23 = 22 + X (Parida & Sharma 1987; Sharma & Parida 1987); *Physocyclus californicus* Chamberlin & Gertsch 1929, 2n (♂) = 15 = 14 +X (Cokendolpher 1989); *Physocyclus enaulus* Crosby 1926, 2n (♂) = 15 = 14 + X (Cokendolpher 1989) and *Physocyclus* sp., 2n(♂) = 15 = 14 + X (Cokendolpher & Brown 1985; Cokendolpher 1989). In the species whose chromosomal morphology has been determined, all chromosomes are metacentric, with the exception of the X_1 and X_2 chromosomes of *C. lyoni* described by Sharma et al. (1959) and *P. crypticolens*, which are acrocentric. There are no cytogenetical data on "ninetines" and "New World clade".

The genus *Mesabolivar* González-Sponga 1998, included in the New World clade by Huber (2000), includes 34 species from which 24 occur in Brazil (Huber 2005). This genus arises as a sister group of *Coryssocnemis* Simon 1893; however, this position is not yet clearly established. The genus *Mesabolivar* has been divided into four "operational" groups, based on morphological characters: a "northern group with spines on male metatarsi" (5 species), a "northern group without spines on male metatarsi" (6 species), a "southern/eastern group" (15 species) probably not monophyletic, and a "miscellaneous group" (7 species), certainly polyphyletic, that will probably be partly transferred to other genera/group (Huber 2000).

Mesabolivar luteus (Keyserling 1891) is a species belonging to the "miscellaneous group," probably related to *Mesabolivar levii* Huber 2000, and is distributed in the states of Rio de Janeiro, São Paulo, Paraná and Rio Grande do Sul, in Brazil. The genus *Micropholcus* Deeleman-Reinhold & Prinsen 1987 (pholcine) includes only two species, of which only the Pantropical species *Micropholcus fauroti* (Simon 1887) occurs in Brazil by introduction and lives as a synanthropic species (Huber 2000).

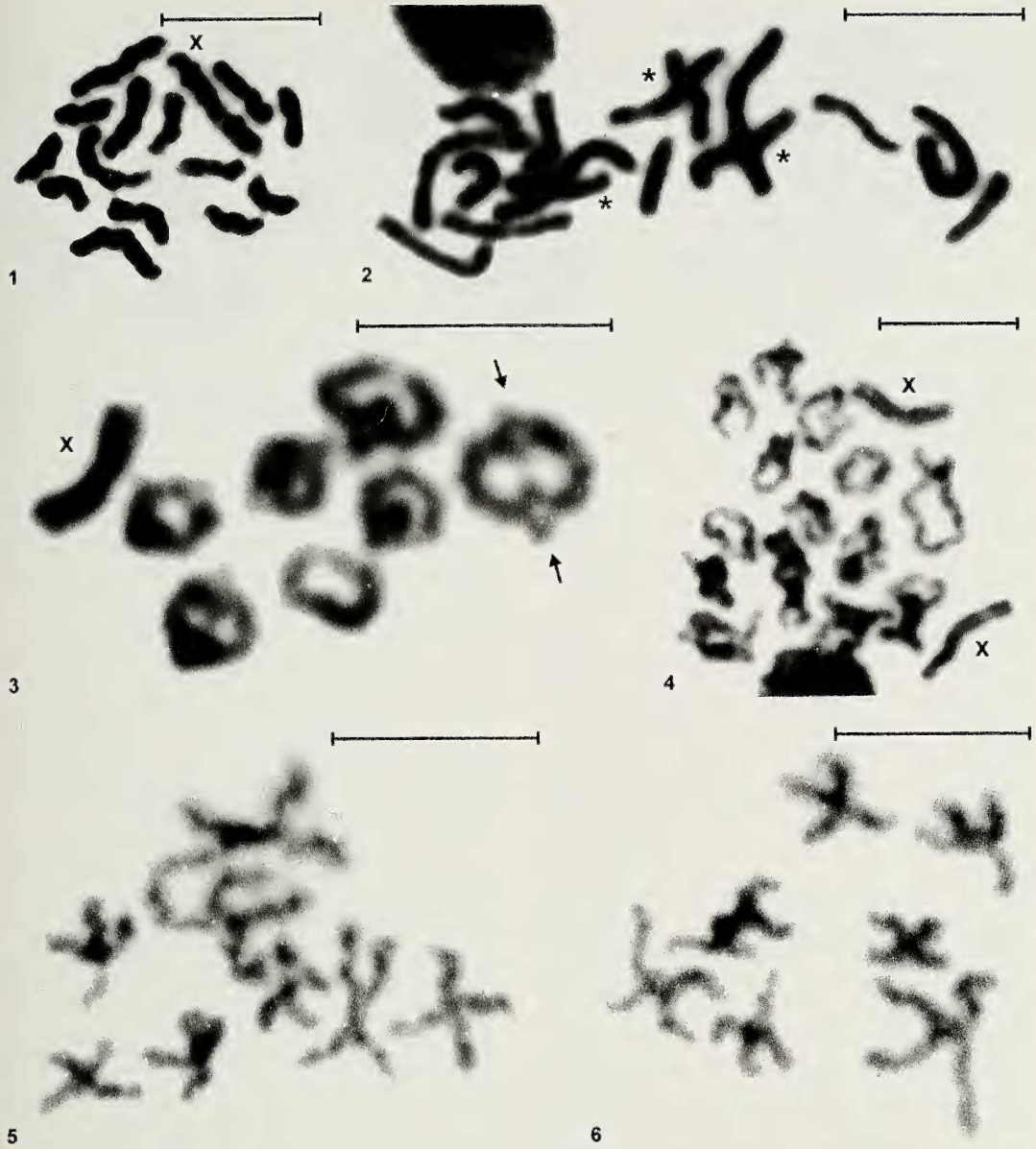
The use of chromosomal data in phylogenetic analysis is relatively new, and the criteria to codify these data are controversial (Modi 1987; Borowik 1995). Additionally, cytogenetic analysis may have some difficulties when compared with other kinds of analysis: the specimens must be kept alive until the slide preparations, some of them do not have cell division at the moment of analysis, and some techniques are expensive. Nevertheless, chromosomal data have a potential usefulness for phylogenetic inference, because they are heritable, homologue states can be identified, and the characters are independent from each other (Borowik 1995). Basically, a chromosomal phylogeny can be constructed based on the minimum number of rearrangements required or the maximum number of shared segments (Rokas & Holland 2000). Although chromosomal data has not been used for cladistic analysis in spiders, there have been some attempts in other groups, such as mammals, to obtain characters by conventional (Nagamachi et al. 1999; Garcia et al. 2000) or molecular cytogenetic techniques (Oliveira et al. 2002).

The aim of this study is to characterize the chromosomes of the species *M. luteus* and *M. fauroti*, analyzing standard stained mitotic and meiotic cells, in order to begin an effort to establish karyotypic relationships among species in the Pholcidae.

METHODS

Three males and one female of *M. luteus* were collected at Maranduba beach, Ubatuba (23°43′S 45°07′W), and five males of *M. fauroti* were collected in buildings in Rio Claro and Ubatuba (22°41′S 47°56′W and 23°43′S 45°07′W), both in the state of São Paulo, southeastern Brazil. The specimens are deposited in the collection of the Laboratório de Artrópodes Peçonhentos, Instituto Butantan, São Paulo (IBSP, A.D. Brescovit) under the numbers IBSP 42785 (*Mesabolivar luteus*), 42782, 42783, 42784, 47504 and 47505 (*Micropholcus fauroti*).

Gonads were dissected in Ringers solution for insects, transferred to colchicine solution (0.16% in Ringer for insects) and left for 2 hrs.; a volume of hypotonic solution (tap water) equal to that of the colchicine solution was added and after 15 mins. the material was placed in Carnoy I fixative solution for 60 min., after which it was macerated in 60% acetic acid on the surface of the slide. The slide was dried on a metal heating plate (35–40 °C) and stained with a 3% Giemsa solution

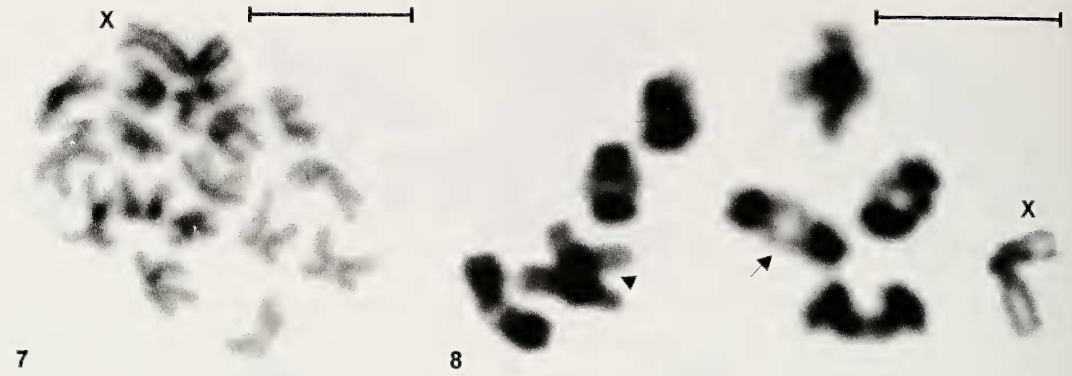


Figures 1–6.—*Mesabolivar luteus* cells. 1. Spermatogonial metaphase, with 2n = 15 = 14 + X. 2. Oogonial metaphase, with 2n = 16 = 14 + XX. The asterisks indicate overlapped chromosomes. 3. Diplotene, with 7II + X. Arrows indicate the chiasma location. 4. Diplotene nuclei, constituting a pair of cells. 5. Metaphase II, with n = 8 = 7 + X. The X could not be identified in this spread. 6. Metaphase II, with n = 7. Scale = 10 μm.

for 13–15 min. The cells were photographed under a Zeiss microscope and the chromosome morphology classification was determined according to Levan et al. (1964). The number of analyzed chromosomal spreads was 65 for *M. luteus* and 40 for *M. fauroti*. In each of these spreads, the chromosome number was determined and no intraspecific variation was detected.

RESULTS

Mesabolivar luteus.—The mitotic metaphases showed 2n = 15 = 14 + X in males (Fig. 1) and 2n = 16 = 14 + XX in females



Figures 7–8.—*Micropholcus fauroti* cells. 7. Spermatogonial metaphase, with 2n = 17 = 16 + X. 8. Diplotene, with 8II + X. Arrow indicates a terminal chiasma and arrowhead points to an interstitial chiasma. Scale = 10 μm.

(Fig. 2). In the spermatogonial metaphases, the X chromosome is always easily identified as the largest element (Fig. 1). The chromosomal morphology is not clear in the mitotic metaphases due to the low degree of chromosome condensation. Diplotene cells showed 7II + X (Fig. 3). All bivalents present a ring shape, evidencing the occurrence of two terminal chiasmata per bivalent, and the X chromosome constitutes an univalent during all meiosis I (Fig. 3). Some diplotene cells appeared in pairs (Fig. 4). Metaphases II showed n = 8 = 7 + X (Fig. 5) or n = 7 (Fig. 6) chromosomes. The X chromosome cannot be recognized in the n = 8 cells due to the irregular chromosome appearance. Despite the low staining contrast, the chromosomal morphology of this species was determined as metacentric.

Micropholcus fauroti.—The spermatogonial metaphases showed 2n = 17 = 16 + X (Fig. 7). The largest chromosome of complement is X, which is easily identified in all analyzed metaphases (Fig. 7). Despite the low staining contrast and the low morphology resolution, the chromosomes seem to be biarmed (Fig. 7). Diplotene cells possessed 8II + X (Fig. 8) and each bivalent shows only one chiasma, terminal or interstitial (Fig. 8). The X chromosome appears as a univalent during meiosis I (Fig. 8).

DISCUSSION

Despite high diversity of pholcid species among haplogynes, this family is poorly known from the cytogenetic point of view. This could be due to the lack of Pholcidae cytogenetic researchers, the relatively small size of pholcid species and their chromosomes, and the difficulty in obtaining good quality chromosomal preparations.

As the generic name suggests, *Micropholcus fauroti* is a very small spider, 1–2mm in length. Thus, dissection of the specimens, as well as the removal of the testis, is very difficult. Additionally, only one slide, with few cells, can be obtained per specimen due to extremely minute size of the testis.

In relation to the chromosome length, Painter (1914), Suzuki (1954) and Bole-Gowda (1958) emphasized the very small size of the elements. The largest chromosome of *P. crypticolens*, obtained by Suzuki (1954), measured only around 2.4 μm. The largest chromosome of *C. lyoni* is the X chromosome, which measures 5.8 μm, but the largest autosome measures only around 2.3 μm (Bole-Gowda 1958). The measurements of the largest chromosomes of *M. luteus* and *M. fauroti* were respectively 9 and 7 μm (for the X chromosome), and 6 and 5 μm (for the autosomes). Thus, the chromosomes of the studied species are not as small as those obtained by Suzuki (1954) and Bole-Gowda (1958). On the other hand, they are not as large as those of other haplogyne genera, such as *Loxosceles* Heineken & Lowe 1832 (Sicariidae) in which the largest chromosomes measure around 15 μm (Silva et al. 2002).

Concerning the preparation quality, Painter (1914) and Suzuki (1954), using different types of fixative solutions, called attention to the unfavorable fixation of pholcid chromo-

somes. A similar problem occurred with *M. luteus* and *M. fauroti* chromosomes, when Carnoy I fixative solution was used, resulting in low staining contrasts. Alternative fixation methods should be tested in pholcid species.

Mesabolivar luteus is the first cytogenetically studied species from the "New World clade" and showed a diploid number equal to that found in three *Physocyclus* species (holocnemines) analyzed by Cokendolpher (1989), despite the fact that these genera belong to different clades. Thus, the 2n = 15 could have arisen independently at least two times within the pholcids. *Micropholcus fauroti* is the first cytogenetically analyzed species from this genus and until now, its diploid number had not yet been recorded in Pholcidae. The presence of biarmed chromosomes in both species of this study is a feature shared among most of the haplogyne group species, as stated by Rodríguez-Gil et al. (2002).

In both species, the largest chromosome of the complement is the X chromosome. This is in agreement with the data obtained by Bole-Gowda (1958) for *C. lyoni* and by Cokendolpher (1989) for three *Physocyclus* species. During interphase, the observed X chromatin positive heteropycnosis of *M. fauroti* is similar to that recorded for *C. lyoni* by Bole-Gowda (1958).

The studied species showed significant differences from each other in relation to the chiasma number, during meiosis. However, information on chiasma number and position was not provided by previous papers on pholcid cytogenetics. Thus, these characteristics cannot be used as parameters to compare related species or to establish a pattern within the pholcid groups.

The diplotene pairs found in *M. luteus* are probably a consequence of the germ cell arrangement and interaction, which constitute "cysts" with synchronously dividing cell connections via intercellular bridges due to the lack of cytokinesis during spermatogenesis. Alberti & Weinmann (1985) described the presence of similar cysts in the testis of *P. phalangiodes*.

In relation to these grouped cells, two questions are crucial: why do they appear in pairs and not in larger groups of cells; and why do these pairs only appear in the diplotene phase? Concerning the first question, Pepling & Spradling (1998) have verified a tendency towards the increase in number by the power of two in mouse embryo oogonial mitotic cells, being more frequently found in clusters of two cells. Clusters with more cells are probably more susceptible to breaks during slide preparation. However, the possibility of finding such clusters in future analysis cannot be discarded. With respect to the second question, this feature is probably a consequence of the skewed cellular phase ratio in the sample, because from the 105 spreads obtained, only 9 were mitotic metaphases and the others were almost all diplotenes. Possibly, paired mitotic metaphases should be also found in *Mesabolivar luteus*. An ultrastructural analysis of spermatogenesis would be of interest to answer these questions. Additionally, further analysis of other pholcid species is needed to verify whether this pairing of cells also occurs.

The possibility of the occurrence of polyploidy in *M. luteus* was discarded, at least in the first instance, due to two main reasons: the lack of polyploid metaphase II cells (despite the low frequency of cells in this meiotic stage) and the lack of tetravalents or chromosomal chains at meiosis I. The formation of chromosomal chains is not a strict rule in polyploids, but they are frequently observed (John 1990).

The cytogenetic analysis of the pholcines *Leptopholcus* Simon 1893 and *Metagonia* Simon 1893, and of the holocnemines *Smeringopus* Simon 1890, *Holocnemus* Simon 1873 and *Priscula* Simon 1893 seems to be extremely important to establish the karyotypic evolution in these two clades. The cytogenetical study of the ninetines and of the New World clade requires more exhaustive research, considering that only *Mesabolivar* was analyzed and that there are numerous genera belonging to these two clades. Finally, when a full cytogenetical data set becomes available for Pholcidae, it could be used to improve the proposed phylogenetic hypothesis for the family.

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